Food Fortification Initiative. Fortification Matters.
Successful atta flour fortification. Using bioavailable iron compounds to increase the absorption of dietary iron

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Potential food fortification vehicles suitable for resource-poor populations

Industrially processed foods regularly consumed by infants, children, adolescents and women of child-bearing age from lower socioeconomic groups

Mass fortification

Cereals
• wheat and maize flours
• artificial rice grains

Condiments
• salt, sugar
• bouillon cubes
• sauces (soy, fish)
• powdered spice mixes

Targeted fortification

Complementary foods for infants and young children

In-home fortification powders and fat-based spreads for infants and young children, but potential for adolescents, pregnant women

potential for market-driven industrial products, but many families have little or no disposable income and need Food Aid
Common micronutrient deficiencies (due to low intake or bioavailability) that can be prevented by fortified foods

- Fe, Zn, I and Vit. A deficiencies well-documented in infants, children and young women particularly from the low socioeconomic populations

  - Fe, Zn deficiency due to
    - cereal/legume based diets, little animal source foods, fruit, vegetables
    - increased demands for growth
    - increased losses (Fe: menstruation, hookworm; Zn: diarrhea)

  - Iodine deficiency due to
    - low soil I

  - Vit. A deficiency due to
    - few animal source foods, orange/yellow fruits/vegetables
Opportunities and barriers to efficacious food fortification

**Iodised salt and vitamin A fortified cooking oil**
- technically not difficult to manufacture
- efficacy well documented

**Zinc fortification**
- technically not difficult
- absorption decreased by phytic acid
- no reliable measure of zinc status $\rightarrow$ no confirmed efficacy

**Iron fortification**
- WHO Guidelines (2006) give recommendation on compounds and levels for efficacious products
  - technically difficult: highly absorbable compounds lead to sensory changes; no sensory changes with less absorbable compounds
  - absorption decreased by phytic acid and polyphenol compounds
  - infection and inflammation (incl. overweight and obesity) block iron absorption
iron fortification of foods

Iron is the most difficult mineral to add to foods and to ensure adequate absorption.

- Highly absorbable compounds may lead to color and flavor problems.
- Even the highly absorbable compounds may be poorly absorbed.

All major vehicles for iron fortification contain potent absorption inhibitors or are consumed with food containing inhibitors.

Manufacturers must:
- Protect iron from absorption inhibitors, remove them, or
- Adjust the level of iron fortification accordingly.

Organoleptically acceptable compounds are poorly absorbed.
Stages in the development of an iron-fortified food

I. optimize iron compound: highest potential absorption with no organoleptic problems

- select Fe compound with highest relative absorption (WHO Guidelines)

II. optimize absorbed iron to meet consumers need

- make organoleptic trials: colour and taste panels, storage, food processing, home cooking

III. make efficacy study to demonstrate effect on Fe status

- estimate Fe intake \(\Rightarrow\) current need for Fe
- estimate intake of fortification vehicle
- estimate/measure absorption (\(\pm\) enhancer)
  \(\Rightarrow\) define fortification level

monitor prevalence of ID according to WHO/CDC Guidelines
Choice of iron compound

WATER SOLUBLE COMPOUNDS

- Dissolve instantaneously
  - Ferrous sulfate
    - RBV = 100
  - Poorly water soluble/soluble in dilute HCl
- Dissolve completely over time
  - Ferrous fumarate
    - RBV = 100

COMPOUNDS WATER INSOLUBLE/POORLY SOLUBLE IN DILUTE ACID

- Never dissolve completely
  - Electrolytic Fe
    - RBV = ca. 50
  - Ferric pyrophosphate
    - RBV = 15 - 75

Relative bioavailability depends on solubility in gastric juice.

Absolute absorption depends on intake of inhibitors (phytate) and enhancers (ascorbic acid) and iron status of consumer.
Enhancers and inhibitors of iron absorption: Factors which could influence the efficacy of iron fortified foods

**Food factors**

<table>
<thead>
<tr>
<th>Enhancers</th>
<th>Inhibitors</th>
</tr>
</thead>
<tbody>
<tr>
<td>ascorbic acid, also muscle proteins and organic acids</td>
<td>phytic acid (cereals, legumes) phenolic compounds (beans, veg.) milk and legume proteins; calcium</td>
</tr>
</tbody>
</table>

**Iron fortification compounds**

<table>
<thead>
<tr>
<th>Soluble</th>
<th>Poorly soluble</th>
<th>Enhancing</th>
</tr>
</thead>
<tbody>
<tr>
<td>good bioavailability/often poor sensory</td>
<td>less bioavailability/better sensory</td>
<td>NaFeEDTA, iron bisglycinate</td>
</tr>
</tbody>
</table>

**Contamination iron**

- soil, cooking pots, milling

**Subject factors**

- Status of consumer: low status/high absorption
- Other nutrient deficiencies: vitamins A and B₂ (needed to incorporate Fe into Hb)
- Infection and inflammatory disorders, anemia of other causes
- Gut health, gut microflora (?)
Phytic acid inhibits iron absorption

- Atta flour, used to make chapati, roti, naan and puri is whole grain durum wheat flour, high in protein, low in gluten but containing ca. 1% phytic acid
- Phytic acid from atta flour forms a complex with minerals and peptides in the GI tract
- Prevents absorption of Fe, Zn, Ca, Mg
In a bread meal, phytic acid must be drastically decreased before Fe absorption is usefully improved (Hallberg et al. 1989)

4.1 mg Fe/meal
80 g wheat flour extrinsic tag radioiron

<table>
<thead>
<tr>
<th>mg PA/100g</th>
<th>PA:Fe molar ratio</th>
<th>relative Fe abs. %</th>
</tr>
</thead>
<tbody>
<tr>
<td>9</td>
<td>0.2</td>
<td>82</td>
</tr>
<tr>
<td>21</td>
<td>0.4</td>
<td>61</td>
</tr>
<tr>
<td>42</td>
<td>0.7</td>
<td>41</td>
</tr>
<tr>
<td>108</td>
<td>1.7</td>
<td>36</td>
</tr>
<tr>
<td>215</td>
<td>4.5</td>
<td>31</td>
</tr>
<tr>
<td>425</td>
<td>8.8</td>
<td>29</td>
</tr>
<tr>
<td>1080</td>
<td>22.4</td>
<td>18</td>
</tr>
</tbody>
</table>
influence of complete dephytinization on iron absorption from cereal porridges

(Hurrell et al. 2003)

extrinsic tag radioiron, 8-11 adults/study, 50g cereal/300ml water, 2.5mg Fe, phytic acid degraded enzymatically

% Fe abs. (± SE) corrected to low iron status (SF 12µg/L)

<table>
<thead>
<tr>
<th></th>
<th>rice</th>
<th>wheat</th>
<th>maize</th>
<th>high tannin sorghum</th>
</tr>
</thead>
<tbody>
<tr>
<td>PA mg/100g sorghum</td>
<td>160</td>
<td>120</td>
<td>260</td>
<td>870</td>
</tr>
</tbody>
</table>

23.6  26.9  20.1  4.4
Potential ways to increase iron absorption by decreasing the inhibitory effect of phytate in cereals and legumes

**Removal**

- milling cereals (up to ca. 90% removed)
- dialysis/ultrafiltration of protein isolates after acid/salt or alkali treatment

**Enzymatic degradation**

- soaking, germination, fermentation activate the native phytases
- fermentation additionally may provide microbial phytases
- steeping at optimum pH and temperature for maximum phytase activity
- add exogenous phytase during food manufacture
- add exogenous phytase at point of consumption

**Addition of compounds which prevent phytate-mineral binding**

- EDTA and ascorbic acid increase absorption of Fe from high phytate foods. NaFeEDTA and ferrous bisglycinate have 2-3 fold absorption of ferrous sulphate
Phytic acid degradation or ascorbic acid addition improve iron absorption by 5-8 month old infants from soy formula

- Soy formula containing 20 mg Fe/L as ferrous sulfate, 400 mg phytate/L, 110 mg ascorbic acid/L
- Phytate degraded enzymatically < 1.3 mg/L, normal ascorbic acid
- Ascorbic acid increased 220 mg/L, native phytate 400 mg/L
- Iron absorption measured in infants using stable isotopes, paired comparisons

(Davidsson et al. 1994)

![Graph showing iron absorption improvements](image-url)
NaFeEDTA effectively counteracts phytate inhibition

Organoleptic evaluation
- does not provoke fat oxidation in stored cereal flours. May cause colour changes in maize and extruded rice

Relative absorption
- 2-4 times better absorbed than FeSO$_4$ from high phytate meals
- similar or lower absorption than FeSO$_4$ from non-inhibitory meals
- may increase Zn absorption, no effect on Ca or heavy metals
- accepted by JECFA, FDA, EFSA
- ~6 times cost of FeSO$_4$

iron absorption by adults consuming bread rolls with 5 mg Fe (Hurrell et al. 2000)
### WHO Guidelines (2006) for the choice of iron compound in fortified food

**for most vehicles**
the order of preference is:

- ferrous sulphate (FS)
- ferrous fumarate
- encapsulated sulphate or fumarate
- electrolytic iron (2x amount vs. FS)
- ferric pyrophosphate (2x amount vs. FS)
- NaFeEDTA

**for high phytate cereal flours**
and high peptide sauces (soy, fish)

- NaFeEDTA

**for liquid milk products and soft drinks**

- ferrous bisglycinate
- micronized ferric pyrophosphate
- ferric ammonium citrate

**for infant foods**
and open-market foods add ascorbic acid as an enhancer
at 2:1 molar ratio, for high phytate foods at 4:1
Elemental iron for flour fortification

**Electrolytic iron is the only iron powder recommended**, but only for low extraction flour, not for atta flour. Estimated to be about half as well absorbed as ferrous sulfate. Add **DOUBLE QUANTITY**.

H-reduced Fe, atomized reduced Fe, carbonyl iron and CO-reduced Fe powders are not recommended. Inadequate evidence for absorption and efficacy.

Further studies are needed with H-reduced Fe and carbonyl Fe powders.
Designing efficacious fortified foods

Defining the fortification level of individual micronutrients

• For national programs designed to improve status of critical micronutrients in at risk populations:
  - additional amount of micronutrient consumed should fill the gap between current intake and requirement of targeted population when added to the daily intake of one or more food vehicles

• For Fe specifically: fortification level must be adjusted for relative bioavailability of Fe compound,
  - Compared to ferrous sulphate add: 2x Fe as electrolytic Fe
    2x Fe as FPP
    0.5x Fe as NaFeEDTA,
Confirming the efficacy of iron fortified foods

- Randomised, double blind, controlled design, adequate number of subjects (young women or school aged children with ID)
- 6-9 month feeding needed to change iron status, monitor with WHO recommended iron status biomarkers

Efficacy has been demonstrated for iron fortified wheat flour, atta flour, rice, salt, curry powder, sugar, soy sauce, fish sauce, complementary foods, micronutrient powders and spreads.

- Guidelines based on a review of all published iron efficacy studies in adult women, adolescents and children which monitored Hb or iron status parameters. No infant studies.

- Only randomized controlled studies with adequate description of methodology and clearly defined iron compounds were included.

- All iron fortified food vehicles were included

- All studies were >5 months duration

- Studies with added ascorbic acid were excluded, studies with other added micronutrients were included.
<table>
<thead>
<tr>
<th>Iron compound</th>
<th>Dose mg/d</th>
<th>Subject / vehicle</th>
<th>Length of study / Country</th>
<th>Impact</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>Encapsulated Ferrous sulphate&lt;sup&gt;a&lt;/sup&gt;</td>
<td>11.8</td>
<td>6-15 year old children salt (bread, fava beans)</td>
<td>9 months Morocco</td>
<td>efficacious</td>
<td>Zimmermann (2003)</td>
</tr>
<tr>
<td>Ferrous sulphate</td>
<td>10.3</td>
<td>18-40 year old women wheat flour biscuits</td>
<td>9 months Thailand</td>
<td>efficacious</td>
<td>Zimmermann (2005)</td>
</tr>
<tr>
<td>Ferrous sulphate</td>
<td>11</td>
<td>11-18 year old students wheat flour biscuits</td>
<td>6 months China</td>
<td>efficacious</td>
<td>Sun (2007)</td>
</tr>
<tr>
<td>Encapsulated ferrous sulphate&lt;sup&gt;b&lt;/sup&gt;</td>
<td>7.1</td>
<td>18-35 year old women wheat flour biscuits</td>
<td>5.5 months Kuwait</td>
<td>efficacious</td>
<td>Biebinger (2009)</td>
</tr>
</tbody>
</table>

<sup>a</sup> encapsulated with partially hydrogenated vegetable oil (Balchem)
<sup>b</sup> encapsulated with hydrogenated palm oil; mean particle size ca. 40µm
Fortification of salt with encapsulated ferrous sulphate improves iron status in Moroccan school children

(Zimmermann et al. 2003)

- encaps. FS prevents colour changes
- dietary iron intake 9-15 mg/d, low Fe bioav. (5%), salt intake 7-12 g/d,
- salt provided to households, added to bread, fava beans, olives
- 9 months randomized double blind controlled trial in 2x 180 6-15 yr old school children
- monitor Hb, SF and TfR, ZPP

fortification level defined as 1 mg Fe/g

IDA = low Hb +2/3 abnormal iron status parameters
## Efficacy and effectiveness studies with NaFeEDTA

<table>
<thead>
<tr>
<th>Dose (mg/d)</th>
<th>Subjects/vehicle</th>
<th>Length of study/country</th>
<th>Impact</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>7.1</td>
<td>Both sexes</td>
<td>24 months</td>
<td>Very efficacious</td>
<td>Ballot (1989)</td>
</tr>
<tr>
<td></td>
<td>Aged 10+</td>
<td>South Africa</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Curry powder</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4.6</td>
<td>Both sexes</td>
<td>32 months</td>
<td>Very efficacious</td>
<td>Viteri (1995)</td>
</tr>
<tr>
<td></td>
<td>Aged 1+</td>
<td>Guatemala</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>sugar</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>8.6</td>
<td>Women 17-44</td>
<td>6 months</td>
<td>Moderately efficacious</td>
<td>Thuy (2003)</td>
</tr>
<tr>
<td></td>
<td>Fish sauce</td>
<td>Vietnam</td>
<td></td>
<td></td>
</tr>
<tr>
<td>7.5</td>
<td>Women 16-49</td>
<td>18 months</td>
<td>Very effective</td>
<td>Thuy (2005)</td>
</tr>
<tr>
<td></td>
<td>Fish sauce</td>
<td>Vietnam</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4.9</td>
<td>Both sexes 3+</td>
<td>18 months</td>
<td>Very effective</td>
<td>Chen (2005)</td>
</tr>
<tr>
<td></td>
<td>Soy sauce</td>
<td>China</td>
<td></td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>Both sexes 11-18</td>
<td>6 months</td>
<td>Very efficacious</td>
<td>Sun (2007)</td>
</tr>
<tr>
<td></td>
<td>Wheat flour</td>
<td>China</td>
<td></td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>Children 3-8</td>
<td>5 months</td>
<td>Very efficacious</td>
<td>Andang’o (2007)</td>
</tr>
<tr>
<td></td>
<td>Maize porridge</td>
<td>Kenya</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3.5</td>
<td>Children 3-8</td>
<td>5 months</td>
<td>Moderately efficacious</td>
<td>Andang’o (2007)</td>
</tr>
<tr>
<td></td>
<td>Maize porridge</td>
<td>Kenya</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1.3</td>
<td>Children 6-11</td>
<td>5 months</td>
<td>No effect on iron status</td>
<td>Van Stuijvenberg (2007abstract)</td>
</tr>
<tr>
<td></td>
<td>Brown bread</td>
<td>South Africa</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Fortification of Atta flour with NaFeEDTA improves iron status of Indian School children (Muthayya et al, 2012)

- whole grain ‘atta’ wheat flour fortified with NaFeEDTA at 60 ppm
- 7 month randomized double blind controlled trial in 2 x 200 6-13 yrs old children of low Fe status (SF <20 μg/L)
- 100 g atta flour containing 6 mg Fe as NaFeEDTA fed as chapatis with vegetable dishes 6 d/w
- monitor Hb, SF and TfR
### Efficacy and effectiveness studies with electrolytic iron

<table>
<thead>
<tr>
<th>Dose (mg/d)</th>
<th>Subject/vehicle</th>
<th>Length of study/country</th>
<th>Impact</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>12.5</td>
<td>Women 16-50 Wheat flour</td>
<td>24 months Sri Lanka</td>
<td>No change in Hb</td>
<td>Nestel (2004)</td>
</tr>
<tr>
<td>10</td>
<td>Women 18-50 Wheat flour biscuits</td>
<td>9 months Thailand</td>
<td>Moderately efficacious No change in Hb</td>
<td>Zimmermann (2005)</td>
</tr>
<tr>
<td>3.2</td>
<td>Children 6-11 Brown bread</td>
<td>7.5 months South Africa</td>
<td>No change in iron status</td>
<td>Van Stuijvenberg (2006)</td>
</tr>
<tr>
<td>21</td>
<td>Children 11-18 Wheat flour</td>
<td>6 months China</td>
<td>Moderately efficacious</td>
<td>Sun (2007)</td>
</tr>
<tr>
<td>7</td>
<td>Children 3-8 Maize porridge</td>
<td>5 months Kenya</td>
<td>No change in iron status</td>
<td>Andang’o (2007)</td>
</tr>
<tr>
<td>4.5</td>
<td>Children 6-11 Brown bread</td>
<td>8 months South Africa</td>
<td>No change in iron status</td>
<td>Van Stuijvenberg (2007 Abstract)</td>
</tr>
<tr>
<td>11</td>
<td>Children 6-14 Wheat flour biscuits</td>
<td>6 months Ivory Coast</td>
<td>No change in iron status</td>
<td>Rohner (2010)</td>
</tr>
</tbody>
</table>
Minimum daily amount of iron from different compounds which have been demonstrated to be efficacious in women

- Ferrous sulfate, 7.1 mg - 4 studies, all efficacious
  NaFeEDTA, 4.6mg - 10 studies, 9 efficacious
  Electrolytic iron 10mg - 7 studies, 2 efficacious

- Evidence-based values from studies with a demonstrated decrease in prevalence of ID/IDA.
Recommendations for iron fortification of wheat flour

<table>
<thead>
<tr>
<th>Type of extraction</th>
<th>Iron Fortificant</th>
<th>Fortification level (mg/kg) by per capita flour intake (/d)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>&lt;75g/d</td>
</tr>
<tr>
<td>Low (low PA)</td>
<td>NaFeEDTA</td>
<td>40</td>
</tr>
<tr>
<td></td>
<td>Sulphate/fumarate</td>
<td>60</td>
</tr>
<tr>
<td></td>
<td>Electrolytic Fe</td>
<td>NR</td>
</tr>
<tr>
<td>High (high PA)</td>
<td>NaFeEDTA</td>
<td>40</td>
</tr>
</tbody>
</table>

- NaFeEDTA is the only iron compound demonstrated to be efficacious in atta flour.

- Ferrous sulphate/fumarate not tested. Would need to be added at 2-3 times Fe level of NaFeEDTA. Sensory changes are likely.